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RELATION OF DIFFERENT STRAINS OF INFLUENZA BACILLI AS SHOWN BY CROSS AGGLUTINA- TION AND ABSORPTION TESTS

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Great endeavor has been made to throw light on the obscure problems relative to the etiology of influenza. Investigation of this character was more general during the epidemic occurring early in 1920 than during the greater one of the previous year, although the opportunity to observe patients suffering from this malady lasted over a much briefer period. In association with each epidemic much interest has been taken in the small gram-negative bacillus described by Pfeiffer¹ as *Bacillus influenzae*. Little by little knowledge is accumulating which it is hoped will show what relation this organism may have to the disease.

In anticipation of the epidemic that occurred early in 1920, a study of the influenza bacillus was commenced late in October, 1919. A survey was made to determine the incidence of the bacillus in the upper respiratory tract of healthy medical students from different localities of the Middle West.

Great stress was laid on the technic in this survey. The swabs were prepared from fairly heavy copper wire with the terminal 15 mm. bent to an angle of approximately 135 degrees to facilitate swabbing high in the pharynx. Cultures made from the pharynx gave more positive results than those from the tonsillar regions. The advantage gained by swabbing the pharynx had been pointed out by Winchell and Stillman.²

The medium employed was meat infusion 2% agar to which 4% defibrinated rabbit blood was added. Slightly more or less blood made no appreciable difference in the degree of growth, while marked divergence showed less favorable results. Four per cent, seemed to be the mean point of maximum growth. Brown blood was made by heating the medium to approximately 80 C. sufficiently long to pro-

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¹ Ztschr. f. Hyg. u. Infektionskrankh., 1893, 13, p. 357.

² Jour. Exper. Med., 1919, 30, p. 497.

duce a distinct brown color without a trace of red. The most desirable hydrogen-ion concentration was thought to be P_H 7.3 or 7.4. Here again organisms that grew well bore considerable variation of reaction. Nevertheless, P_H 7.3 or 7.4 represented the mean point of alkalinity showing maximum growth. It is of interest that Avery³ used a medium with a final P_H of 7.3 to 7.5, while Winchell and Stillman used medium with a P_H of 7.2 to 7.5. In the first part of my work red blood medium was used exclusively although later more brown blood medium was employed. Brown blood was preferred because the colonies grew more luxuriantly and were considered as characteristic to those experienced as the so-called dew-drop colonies on red blood agar.

Petri dishes were prepared and inoculated along one border from the sides of the swab by rolling it on the surface of the agar, and from this inoculated area the organisms were spread over the remaining surface of the plate with a platinum needle. The area of primary inoculation from the swab always showed a confluent growth, from which small gram-negative bacilli were demonstrated in every instance in a stained film. From 10-30 colonies were picked from the sparsely inoculated area in the endeavor to isolate the small gram-negative bacillus observed in the film. A Petri dish was divided into small squares by a glass-pencil. The colonies picked from the throat culture were inoculated into these squares and incubated 24 hours. This gave sufficient material from each colony picked to avoid losing it, and when colonies were crowded avoided contamination to a great extent. This method was fairly rapid. If the organism was not isolated from the first throat culture, subsequent cultures were made and in a few instances as many as 5 cultures were taken from the same throat. A series of 28 was studied. Failure to isolate the organism was recorded in one instance after 7 attempts, although in the stained film a few gram-negative bacilli were observed. These were accepted as influenza bacilli, if they failed to grow on meat infusion 2% agar of desirable hydrogen-ion concentration (to which 4% ascitic fluid was added) and if they grew well when 4% defibrinated blood was added.

Winchell and Stillman studied the incidence of the influenza bacillus in normal throats by reculturing once a month from December, 1918, to May, 1919, inclusive. The percentage of positive results varied from 13 to 51%.

³ Jour. Amer. Med. Assn., 1918, 71, p. 2050.

Jordan⁴ found that 10 of 13 strains of the influenza bacillus studied by him had the property of producing indol. By plating he found the organism in 40% of normal throats. However, by testing for indol in association with mixed cultures he believes that its presence was demonstrated in 80% of his series.

The bacillus of influenza was found in the mouths of 35.1% of all healthy men examined at Camp Funston during the autumn of 1918 by Opie⁵ and his associates.

Lord⁶ and his co-workers made cultures from the throats of 34 healthy Harvard students at an army training camp in order to find the bacillus of influenza and found 76% of the cultures were positive. They give a table showing the occurrence of the influenza bacillus in association with diseases other than influenza which shows that they were present in from 19 to 100% of cases in tuberculosis, measles, varicella, etc.

Park⁷ points out the importance of careful selection of material for culture, good medium and long training in the study of colonies. Too great emphasis cannot be laid on his comment.

It must be remembered that the survey under consideration was made on the crest of an epidemic and merged into a study of the incidence of the influenza bacillus in the throats of influenza patients.

The bacillus of influenza was isolated from the throats of all patients from whose throats I made cultures, the material being selected from patients in the St. Louis Children's Hospital. For a time the work was carried on by Dr. Park J. White, and at the beginning of his work he obtained positive cultures in about 30%. This figure rose as experience and familiarity with the technic increased, until he also was able to isolate the organism from 100% of influenza patients. On recultivating the cases still accessible from which negative results had previously been obtained, he was able to isolate the organism in each instance.

Opie⁸ and his associates, in the report on pneumonia following influenza at Camp Pike, stated that the influenza bacillus was invariably present in the upper respiratory tract of patients with influenza.

Park⁷ states that Williams cultivated 278 cases for the influenza bacillus with these results:

Hospital cases	80% positive
Marines	100% positive
Home for Children.....	98% positive

Pritchitt and Stillman⁹ studied the incidence of the influenza bacillus and the pneumococcus in association with influenza and pneumonia and found:

Normal	42% positive
Convalescent from influenza.....	46% positive
Uncomplicated influenza	83% positive
Influenza with bronchopneumonia.....	93% positive
Bronchopneumonia	100% positive
Lobar pneumonia	55% positive

⁴ Jour. Am. Med. Assn., 1919, 72, p. 1542.

⁵ Ibid., p. 108.

⁶ Ibid., p. 188.

⁷ Ibid., 1919, 73, p. 318.

⁸ Ibid., 72, p. 556.

⁹ Jour. Exper. Med., 1919, 29, p. 259.

Winchell and Stillman found 39% positive in an asylum for boys in which no influenza occurred; 38% were positive among 52 convalescents from influenza in a home for girls. Jordan¹⁰ found that 64% of cases cultivated by him between October, 1918, and February, 1919, gave positive results.

No attempt is made to review the literature on the incidence of the influenza bacillus in the normal throat, its incidence in association with other diseases or in association with influenza. Sufficient is quoted, however, to show the variation in results obtained.

A small diplostreptococcus resembling those studied especially by Tunnicliff¹¹ was observed in a number of instances. No special study was made of this organism.

The morphology of the influenza bacillus varied considerably; still it may be stated that 3 main types were observed: The more usual type was chiefly composed of small bacilli, which were fairly uniform in size and contour. A few or many forms showing pleomorphism were usually present. Coccoid forms, bacilli with one end broader than the other, some large bacilli, and heavy polar staining were observed. The pleomorphism varied to some extent in degree and type, depending on the age of the culture and the medium employed. No leptothrix forms have been observed in these strains after between 4 and 6 months' cultivation on brown blood medium. The second type was a small bacillus which was uniform in size and contour showing no pleomorphism. A subdivision of this type was made; these strains showed long slender filaments or leptothrix forms which manifested greater or less capacity for division. The filaments were of uniform diameters. In certain instances the filaments completely disappeared and only short bacilli of uniform size and contour were observed, in other instances, however, filaments recurred. The filaments at times showed unilateral or bilateral depressions on the sides, which seemed to indicate an attempt at division. A third type is represented by fairly long slender bacilli with slightly pointed ends and at times slightly curved. A few moderately long forms were seen, but as a rule the size was rather uniform. No very short forms were observed. Two strains of this type were studied and showed another peculiarity referred to later.

After long cultivation little change in the organism and cultures was observed; however, the growth was more luxuriant. Most strains produced a grayish growth on brown blood agar and the so-called "dewdrop" colonies when transferred to red blood agar. A few strains

¹⁰ Jour. Infect. Dis., 1919, 25, p. 28.

¹¹ Ibid. 1920, 26, p. 405.

produced a colorless growth on brown blood agar, although in several instances a slightly grayish color appeared in older cultures. All the strains required hemoglobin in the medium. No distinct and constant changes on the blood were produced by their growth. The organisms grew better aerobically and in fresh medium which was not dried.

Pfeiffer's "*Bacillus influenzae*" was gram-negative; the bacilli were small and forms in division resembled cocci. Long filaments were observed and considered as involution forms. The organism was nonmotile and noncapsulated. He later isolated very long forms from a child with bronchopneumonia following diphtheria, which he was unable to differentiate from the former organism and designated it as "*Bacillus pseudo-influenzae*."

Four years later Grassberger¹² studied the morphology of this organism and recorded short forms and filamentous forms. Sometimes the poles of the bacillus showed greater affinity for stain than the body. He believed that the age of the culture and reaction of the medium influenced morphologic changes.

Wollstein¹³ divided her cultures into two chief types; short forms, showing pleomorphism and at times polar staining, and long forms showing a greater or less degree of division.

Galli Valerio¹⁴ discussed involution forms of this organism. He mentioned ovoid forms, spherical forms, clubbed forms which are often in juxtaposition, forms showing irregular staining, and long forms. These changes were observed especially in old cultures.

In 5 cases in my series colonies were picked from the same plate which fulfilled the requirements of the influenza bacillus, but which, however, differed in morphology. These strains were investigated by agglutination and absorption tests in 3 instances.

Twenty-seven rabbits were immunized and bled when the preliminary titration of the serum was satisfactory, that is, above 1,600.

The strains selected were from normal throats in 5 cases and from the throats of influenza patients in 22. Nos. 2, 3, 4, 5 and 10 came from throats of students who had never had influenza. Nos. 18 and 29 were isolated from influenza suspects. The rest were isolated from typical influenza cases and as a rule early in the development of the disease, although in a few instances pneumonia was developing.

The initial dose proved fatal to several rabbits within an hour or two. Parker¹⁵ and others have demonstrated the toxicity of these organisms for laboratory animals. The most toxic strain was No. 4.

¹² Ztschr. f. Hyg. u. Infektionskrankh., 1897, 25, p. 453.

¹³ Jour. Exper. Med., 1915, 22, p. 445.

¹⁴ R  v. m  d. de la Suisse Rom., 1919, 6, p. 265.

¹⁵ Jour. Immunol., 1919, 4, p. 331.

This organism was isolated from a normal throat of a student who never had influenza, fully 6 weeks before the onset of the epidemic in this vicinity.

Each serum was titrated with 36 organisms in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200 and 1:6,400. In a few instances other dilutions were added. Control emulsions were always incubated. Emulsions of organisms were made by washing a 24-hour growth from the surface of a brow blood agar slant or Petri dish with salt solution. Nos. 29, 26, 31 and 32 gave difficulty. The first of these showed auto-agglutination in the control. In the remaining 3 the growth was cohesive and resisted breaking up into a fine suspension. The last two organisms are the ones described under the discussion of the morphology as type 3. This resistance to emulsification was constant; satisfactory results were obtained by using an 18 or 24 hour-growth on several freshly prepared moist plates. This suspension was shaken for 20 minutes and centrifuged sufficiently long to throw down the larger particles, leaving the finer ones in suspension. The same technic applied to No. 29 was also satisfactory. The agglutinating tubes were incubated at 53 C. during 18 or 20 hours and read macroscopically.

It should be observed on chart 1 that cross agglutination occurred frequently in low dilutions and in a few instances in fairly high dilutions. Some serums have a wider latitude of agglutination than others. Some organisms have a greater susceptibility to agglutination than others. Perhaps this is explained by group relationship.

Absorption tests were employed in those instances which showed cross agglutination in a dilution of 1:800 or above. A 24-hour growth on 3 to 6 Petri dishes was washed off the agar with a dilution of 1:25 of the serum to be absorbed. The mixture was incubated at 53 C. for 4 hours, shaken well at 15 minute intervals, and then left to stand over night in the refrigerator. The clear serum from the top was pipeted off with a capillary pipet. The absorbed serum represented a dilution of 1:25. Some of the unabsorbed serum of the original dilution was kept in the incubator 4 hours at 53 C. and in the refrigerator over night; it was titrated with its own organism as a control and the result is recorded on chart 2 following the series of absorption tests for which it serves as control.

It should be observed that these organisms vary markedly in their capacity to absorb specific agglutinin from a foreign serum.

TABLE 1

AGGLUTINATION BY MONOVALENT IMMUNE SERUM WITH DIFFERENT STRAINS OF B. INFLUENZAE

Numbers Indicate the Highest Dilution in which Agglutination was Present.

Immune serum.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Organisms	Dewey	Ksersman	Ferguson	Dock	Lasersohn	Parsons	B. Ouslander	R. Ouslander	Bell	Shindlewolf	Gerhart	No. 198	Burdlesley	No. 257	No. 219	Cotton	Whitaker	Bunyard	No. 226	No. 240	Hirst 1	Hirst 2	Forella 1	Forella 2	Budrovitch 1	Budrovitch 2	Budrovitch 3
1 Dewey.....	3200	50	400	100	25	25	0	100	200	0	0	50	50	100	200	100	200	25	200	0	0	25	...	0	0	200	0
2 Ksersman.....	7000	50	500	200	50	50	200	100	0	0	50	400	100	400	50	0	0	25	0	100	...	0	25	25	0	400	0
3 Ferguson.....	100	100	5000	800	400	200	200	200	200	50	400	400	100	800	100	25	50	25	0	800	...	200	50	50	100	0	0
4 Dock.....	800	100	400	3200	0	50	25	50	200	0	50	50	100	800	100	25	25	25	25	50	...	800	800	800	200	0	0
5 Lasersohn.....	200	50	400	2000	2000	800	50	50	800	100	400	200	400	200	50	25	100	0	0	800	...	50	100	100	50	0	0
6 Parsons.....	50	500	500	700	2000	3200	3200	6400	100	25	1600	50	400	400	100	50	200	0	25	100	...	0	100	100	25	0	0
7 B. Ouslander.....	200	100	100	200	25	200	6400	6400	200	0	800	100	800	200	100	50	400	25	100	100	...	1600	100	100	50	0	0
8 R. Ouslander.....	100	200	200	100	100	50	800	800	3200	25	800	400	400	50	50	0	0	0	400	800	...	0	50	50	0	0	0
9 Bell.....	100	0	0	0	0	0	0	0	0	2300	2400	800	0	0	0	50	50	0	0	100	...	0	0	0	0	25	0
10 Shindlewolf.....	0	0	0	0	0	0	0	0	0	0	0	0	800	800	200	0	100	25	800	100	...	200	400	800	0	0	0
11 Gerhart.....	100	100	200	400	50	100	400	400	800	25	2400	800	0	0	50	0	100	0	0	100	...	0	0	0	0	0	0
12 No. 198.....	0	0	50	25	25	25	0	0	200	25	100	200	6400	50	0	25	0	0	400	100	...	25	25	25	400	100	0
13 Burdlesley.....	0	0	100	25	200	0	25	25	800	50	800	800	400	800	100	50	0	0	0	200	...	1600	800	800	200	25	0
14 No. 257.....	50	50	800	200	400	200	100	100	200	25	100	400	400	800	800	800	200	50	50	1600	...	25	25	25	25	25	0
15 No. 219.....	200	50	200	800	400	200	25	50	200	50	400	800	100	800	400	6400	100	25	50	800	...	25	50	50	400	50	0
16 Cotton.....	200	0	200	800	200	50	25	50	200	0	0	800	0	0	0	0	6400	0	0	800	...	0	0	0	0	0	0
17 Whitaker.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6400	0	0	0	...	25	0	0	0	0	0
18 Bunyard.....	25	0	200	0	100	0	50	0	200	0	0	0	400	50	0	0	0	3200	0	50	...	400	200	200	400	25	0
19 No. 226.....	50	0	50	200	50	25	100	200	800	100	200	800	200	800	800	25	50	200	6400	200	...	400	50	50	800	100	0
20 No. 240.....	200	50	100	50	800	100	50	50	100	50	100	200	200	800	0	0	400	0	800	6400	...	1600	400	0	0	0	0
21 Hirst 1.....	0	0	0	25	0	0	0	0	200	0	0	200	0	0	0	0	0	25	0	3200	...	1600	0	0	0	0	0
22 Hirst 2.....	0	0	0	0	0	0	0	0	200	0	0	200	0	0	0	0	0	25	0	3200	...	1600	0	0	0	0	0
23 Forella 1.....	25	100	200	100	25	100	50	50	0	0	400	50	200	400	25	0	0	0	50	100	...	25	6400	3200	0	25	0
24 Forella 2.....	25	100	400	300	100	50	100	100	0	0	200	100	400	400	25	50	0	25	50	100	...	25	3200	3200	25	0	0
25 Budrovitch 1.....	25	200	800	100	800	400	0	25	200	25	400	200	400	400	0	0	25	0	50	400	...	200	100	3200	400	0	0
26 Budrovitch 2.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	...	0	0	0	3200	0	0
27 Budrovitch 3.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	...	0	0	0	0	6400	0
28 Fitzporter.....	0	0	0	0	0	0	0	0	0	25	0	0	800	800	400	0	25	0	400	0	...	200	400	400	400	0	0
29 Smith.....	0	50	0	100	200	200	100	200	800	800	800	400	200	0	25	0	200	200	200	0	...	25	25	25	25	0	0
30 Meyer.....	0	200	200	400	200	100	200	400	800	800	800	200	0	0	50	25	0	0	0	50	...	0	0	0	0	0	0
31 Sehnard.....	0	100	0	400	0	50	0	0	0	0	25	0	0	0	100	50	0	0	0	0	...	0	0	400	0	0	0
32 Solmski.....	25	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	800	0	0	0	...	0	0	0	0	0	0
33 V. Brooks.....	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	...	25	800	800	400	25	0	0
34 Stoute.....	100	50	200	200	400	50	100	50	800	200	200	100	400	400	50	0	50	1600	0	800	...	800	100	400	400	0	0
35 Brady.....	200	25	200	200	200	400	100	100	800	50	400	100	200	200	50	25	0	0	1600	800	...	0	100	400	400	0	0
36 Fenton.....	50	0	0	200	0	0	25	100	0	0	0	0	0	0	0	0	0	25	0	0	...	0	25	25	0	0	0
Immune serum.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27

Where absorption occurred these tests were repeated two and occasionally three times to cause complete absorption if possible. The results were confirmative of the first observations. In a few instances the titer of the control was lower than the original titer of the serum. Diluted serum of several days' standing was occasionally used and the titer of this was often diminished. This made no difference, however, as the control and the absorbed serum were of the same dilution.

When a serum was absorbed by a foreign organism, this organism absorbed all, part, or none of the agglutinins for other foreign strains so tested. This was subject to considerable variation.

It should be observed that B. Ouslander and R. Ouslander were identical strains. These were isolated from two children of the same family suffering from influenza. Hirst 1 and Hirst 2 strains isolated from the same plate, showing marked difference in morphology, were nevertheless identical strains. Fiorella 1 and 2 were isolated from the same plate and showed a marked difference in morphology; these were identical strains. On the contrary, Budrovich 1, 2 and 3, isolated from the same plate and showing marked difference in morphology, were proved to be distinct types, as shown by these tests.

The serum of No. 5, agglutinated organisms 5 and 6 in equally high dilution. By absorption tests it was shown that these organisms were not identical. No 25 had the capacity to absorb nearly all the specific agglutinins from serum of No. 5. This test was repeated 3 times, using a greater excess of organisms, with uniform result. This relation was not reciprocal. They were not identical strains. No grouping into types can be recognized on these charts. However, certain strains are closer related than others and can be divided into subtypes to a limited extent. For example, Nos. 5 and 6, also 9 and 19, show considerable cross agglutination. Many other strains show a close relation to these subtypes, which adds difficulty to separating them into distinct subgroups.

Two additional strains were studied later and not included in the charts. One was isolated from the lung of an adult with lobar pneumonia caused by the pneumococcus type 2. The second was isolated from a child suffering from bronchopneumonia with abscess formation following measles. *Streptococcus hemolyticus* and *Micrococcus aureus* were also isolated from the areas of consolidation. These strains resembled the organism included in the table and in no instance were they agglutinated by dilutions above 1:800. No absorption

TABLE 2
CROSS AGGLUTINATION AFTER ABSORPTION TESTS

Immune Serum for Organism	Absorbed by Organism	Titred with Organism	Titer	Immune Serum for Organism	Absorbed by Organism	Titred with Organism	Titer
No. 1				B. Ouslander	B. Ouslander	Brady	0
Dewey	Dewey	Dewey	0	B. Ouslander	R. Ouslander	R. Ouslander	0
Dewey	Dewey	Dock	0	B. Ouslander	R. Ouslander	B. Ouslander	0
Dewey	Dock	Dock	0	B. Ouslander	R. Ouslander	Brady	0
Dewey	Dock	Dewey	800	B. Ouslander	Bell	Bell	0
Dewey	Unabsorbed (control)	Dewey	1600	B. Ouslander	Bell	B. Ouslander	800
No. 3				B. Ouslander	Brady	Brady	0
Ferguson	Ferguson	Ferguson	0	B. Ouslander	Brady	B. Ouslander	1200
Ferguson	Ferguson	Parsons	0	B. Ouslander	Brady	R. Ouslander	800
Ferguson	Ferguson	Lasersohn	0	B. Ouslander	Brady	R. Ouslander	1600
Ferguson	Ferguson	219	0	B. Ouslander	Unabsorbed (control)	B. Ouslander	0
Ferguson	Ferguson	Budrovitch 1	0	No. 8			
Ferguson	Ferguson	Bell	0	R. Ouslander	R. Ouslander	R. Ouslander	0
Ferguson	Parsons	Parsons	0	R. Ouslander	R. Ouslander	B. Ouslander	0
Ferguson	Parsons	Ferguson	3200	R. Ouslander	B. Ouslander	B. Ouslander	0
Ferguson	Lasersohn	Lasersohn	0	R. Ouslander	Unabsorbed (control)	R. Ouslander	1600
Ferguson	Lasersohn	Ferguson	3200	No. 9			
Ferguson	219	Ferguson	3200	Bell	Bell	Bell	0
Ferguson	219	Ferguson	3200	Bell	Bell	Lasersohn	0
Ferguson	Brady	Brady	0	Bell	Bell	Gerhart	0
Ferguson	Brady	Ferguson	3200	Bell	Bell	257	0
Ferguson	Budrovitch 1	Budrovitch 1	0	Bell	Bell	226	0
Ferguson	Budrovitch 1	Ferguson	3200	Bell	Bell	Brady	50
Ferguson	Unabsorbed (control)	Ferguson	3200	Bell	Bell	Meyer	0
No. 4				Bell	Lasersohn	Lasersohn	0
Dock	Dock	Dock	0	Bell	Lasersohn	Bell	2400
Dock	Dock	Ferguson	0	Bell	Lasersohn	Brady	800
Dock	Dock	Lasersohn	0	Bell	Gerhart	Gerhart	0
Dock	Dock	219	0	Bell	Gerhart	Bell	1600
Dock	Dock	Cotton	0	Bell	226	226	0
Dock	Ferguson	Ferguson	0	Bell	226	Bell	800
Dock	Ferguson	Dock	800	Bell	226	Meyer	0
Dock	Lasersohn	Lasersohn	0	Bell	Brady	Brady	0
Dock	Lasersohn	Dock	800	Bell	Brady	Bell	800
Dock	219	219	0	Bell	Brady	Lasersohn	0
Dock	219	Dock	800	Bell	Meyer	Meyer	0
Dock	Cotton	Cotton	0	Bell	Meyer	Bell	1600
Dock	Cotton	Dock	400	Bell	Meyer	226	400
Dock	Unabsorbed (control)	Dock	1600	Bell	257	257	0
No. 5				Bell	257	Bell	1600
Lasersohn	Lasersohn	Lasersohn	0	Bell	Unabsorbed (control)	Bell	2400
Lasersohn	Lasersohn	Parsons	0	No. 11			
Lasersohn	Lasersohn	240	0	Gerhart	Gerhart	Gerhart	0
Lasersohn	Lasersohn	Budrovitch 1	0	Gerhart	Gerhart	B. Ouslander	0
Lasersohn	Lasersohn	Stolte	0	Gerhart	Gerhart	R. Ouslander	0
Lasersohn	Lasersohn	Ferguson	0	Gerhart	Gerhart	Bell	0
Lasersohn	219	219	0	Gerhart	Gerhart	257	0
Lasersohn	219	Lasersohn	400	Gerhart	Gerhart	Meyer	0
Lasersohn	Parsons	Parsons	0	Gerhart	B. Ouslander	B. Ouslander	0
Lasersohn	Parsons	Lasersohn	400	Gerhart	B. Ouslander	Gerhart	400
Lasersohn	240	240	0	Gerhart	R. Ouslander	R. Ouslander	0
Lasersohn	240	Lasersohn	800	Gerhart	R. Ouslander	Gerhart	800
Lasersohn	Budrovitch 1	Budrovitch 1	0	Gerhart	Meyer	Meyer	0
Lasersohn	Budrovitch 1	Lasersohn	50	Gerhart	Meyer	Gerhart	100
Lasersohn	Budrovitch 1	240	400	Gerhart	Unabsorbed (control)	Gerhart	1600
Lasersohn	Unabsorbed (control)	Lasersohn	1600	Gerhart	Bell	Bell	0
No. 6				Gerhart	Bell	Gerhart	1600
Parsons	Parsons	Parsons	0	Gerhart	257	257	0
Parsons	Parsons	Lasersohn	0	Gerhart	257	Gerhart	3200
Parsons	Lasersohn	Lasersohn	0	Gerhart	Unabsorbed (control)	Gerhart	3200
Parsons	Lasersohn	Parsons	3200	No. 12			
Parsons	Unabsorbed (control)	Parsons	3200	198	198	198	0
No. 7				198	198	B. Ouslander	0
B. Ouslander	B. Ouslander	B. Ouslander	0	198	198	Bell	0

RELATION OF DIFFERENT STRAINS OF INFLUENZA BACILLI 473

TABLE 2—Continued
CROSS AGGLUTINATION AFTER ABSORPTION TESTS

Immune Serum for Organism	Absorbed by Organism	Titred with Organism	Titer	Immune Serum for Organism	Absorbed by Organism	Titred with Organism	Titer
B. Ouslander	B. Ouslander	R. Ouslander	0	No. 16			
B. Ouslander	B. Ouslander	Bell	0	Cotton	Cotton	Cotton	0
198	198	257	0	Cotton	Cotton	219	0
198	198	Cotton	0	Cotton	219	219	0
198	198	Gerhart	0	Cotton	219	Cotton	800
198	198	226	0	Cotton	219	R. Ouslander	200
198	Bell	Bell	0	Cotton	Unabsorbed (control)	Cotton	800
198	Bell	198	2400				
198	B. Ouslander	B. Ouslander	0	No. 17			
198	B. Ouslander	198	1600	Whitaker	Whitaker	Whitaker	0
198	Gerhart	Gerhart	0	Whitaker	Whitaker	V. Brooks	0
198	Gerhart	198	2400	Whitaker	V. Brooks	V. Brooks	0
198	257	257	0	Whitaker	V. Brooks	Whitaker	3200
198	257	198	2400	Whitaker	Unabsorbed (control)	Whitaker	3200
198	Cotton	Cotton	0				
198	Cotton	198	1600	No. 18			
198	226	226	0	Bunyard	Bunyard	Bunyard	0
198	226	198	2400	Bunyard	Bunyard	Brady	0
198	Unabsorbed (control)	198	3200	Bunyard	Brady	Bunyard	1600
				Bunyard	Unabsorbed (control)	Bunyard	1600
No. 13							
Burdsley	Burdsley	Burdsley	0	No. 19			
Burdsley	Burdsley	Gerhart	0	226	226	226	0
Burdsley	Burdsley	257	0	226	226	Bell	0
Burdsley	Burdsley	R. Ouslander	0	226	226	Gerhart	0
Burdsley	Burdsley	Brady	0	226	226	198	0
Burdsley	Gerhart	Gerhart	0	226	226	240	0
Burdsley	Gerhart	Burdsley	2400	226	226	Brady	0
Burdsley	257	257	0	226	226	Bell	0
Burdsley	257	Burdsley	2400	226	Bell	226	800
Burdsley	R. Ouslander	R. Ouslander	0	226	Bell	Gerhart	0
Burdsley	R. Ouslander	Burdsley	2400	226	Gerhart	Gerhart	1600
Burdsley	Brady	Brady	0	226	Gerhart	240	50
Burdsley	Brady	Burdsley	2400	226	Gerhart	198	0
Burdsley	Unabsorbed (control)	Burdsley	2400	226	198	226	1600
				226	198	Brady	0
No. 14				226	240	240	0
257	257	257	0	226	240	226	1600
257	257	Ferguson	0	226	240	Gerhart	50
257	257	Dock	0	226	240	Brady	0
257	257	Lasersohn	0	226	Brady	226	1600
257	257	Gerhart	0	226	Brady	198	100
257	257	219	0	226	Unabsorbed (control)	226	1600
257	257	Cotton	0				
257	257	240	0	No. 20			
257	Ferguson	Ferguson	0	240	240	240	0
257	Ferguson	257	3200	240	240	Ferguson	0
257	Ferguson	Dock	200	240	240	Lasersohn	0
257	Dock	Dock	0	240	240	Parsons	0
257	Dock	257	4800	240	240	Bell	0
257	Lasersohn	Lasersohn	0	240	240	Cotton	0
257	Lasersohn	257	4800	240	240	219	0
257	Gerhart	Gerhart	0	240	240	Brady	0
257	Gerhart	257	4800	240	240	Ferguson	0
257	219	219	0	240	Ferguson	240	3200
257	219	257	3200	240	Lasersohn	Lasersohn	0
257	Cotton	Cotton	0	240	Lasersohn	240	800
257	Cotton	257	4800	240	Parsons	Parsons	0
257	240	240	0	240	Parsons	240	100
257	240	257	4800	240	Bell	Bell	0
257	Unabsorbed (control)	257	4800	240	Bell	240	1600
				240	Cotton	Cotton	0
No. 15				240	Cotton	240	1600
219	219	219	0	240	219	219	0
219	219	240	0	240	219	240	1600
219	240	219	3200	240	Brady	Brady	0
219	240	Lasersohn	200	240	Brady	240	1600
219	Unabsorbed (control)	219	3200	240	Unabsorbed (control)	240	3200

TABLE 2—Continued
CROSS AGGLUTINATION AFTER ABSORPTION TESTS

Immune Serum for Organism	Absorbed by Organism	Titrated with Organism	Titer	Immune Serum for Organism	Absorbed by Organism	Titrated with Organism	Titer
No. 21				Fiorella 2	Stolte	Fiorella 2	1600
Hirst 1	Hirst 1	Hirst 1	0	Fiorella 2	Stolte	219	400
Hirst 1	Hirst 1	Hirst 2	0	Fiorella 2	219	219	0
Hirst 1	Hirst 2	Hirst 2	0	Fiorella 2	219	Fiorella 2	1600
Hirst 1	Hirst 2	Hirst 1	0	Fiorella 2	Fiorella 1	Fiorella 1	0
Hirst 1	Unabsorbed (control)	Hirst 1	1600	Fiorella 2	Fiorella 1	Fiorella 2	0
No. 22				Fiorella 2	Fiorella 1	Lasersohn	0
Hirst 2	Hirst 2	Hirst 2	0	Fiorella 2	Unabsorbed (control)	Fiorella 2	1600
Hirst 2	Hirst 2	Hirst 1	0	No. 25			
Hirst 2	Hirst 2	Bell	0	Budrovitch 1	Budrovitch 1	Budrovitch 1	0
Hirst 2	Hirst 2	Lasersohn	0	Budrovitch 1	Budrovitch 1	Lasersohn	0
Hirst 2	Hirst 2	219	0	Budrovitch 1	Budrovitch 1	Parsons	0
Hirst 2	Hirst 2	Brady	0	Budrovitch 1	Budrovitch 1	240	0
Hirst 2	Hirst 1	Hirst 1	0	Budrovitch 1	Budrovitch 1	Budrovitch 2	0
Hirst 2	Hirst 1	Hirst 2	0	Budrovitch 1	Lasersohn	Lasersohn	0
Hirst 2	Bell	Bell	0	Budrovitch 1	Lasersohn	Budrovitch 1	800
Hirst 2	Bell	Hirst 2	3200	Budrovitch 1	Lasersohn	240	400
Hirst 2	Lasersohn	Lasersohn	0	Budrovitch 1	Parsons	Parsons	0
Hirst 2	Lasersohn	Hirst 2	3200	Budrovitch 1	Parsons	Budrovitch 1	800
Hirst 2	219	219	0	Budrovitch 1	240	240	0
Hirst 2	219	Hirst 2	3200	Budrovitch 1	240	Budrovitch 1	400
Hirst 2	Brady	Brady	0	Budrovitch 1	Budrovitch 2	Budrovitch 1	1600
Hirst 2	Brady	Hirst 2	3200	Budrovitch 1	Budrovitch 3	Budrovitch 1	1600
Hirst 2	Unabsorbed (control)	Hirst 2	3200	Budrovitch 1	Unabsorbed (control)	Budrovitch 1	1600
No. 23				No. 26			
Fiorella 1	Fiorella 1	Fiorella 1	0	Budrovitch 2	Budrovitch 2	Budrovitch 2	0
Fiorella 1	Fiorella 1	Fiorella 2	0	Budrovitch 2	Budrovitch 2	B. Ouslander	0
Fiorella 1	Fiorella 2	Fiorella 2	0	Budrovitch 2	Budrovitch 2	B. Ouslander	0
Fiorella 1	Fiorella 2	Fiorella 1	0	Budrovitch 2	Budrovitch 2	Gerhart	50
Fiorella 1	Unabsorbed (control)	Fiorella 1	1600	Budrovitch 2	B. Ouslander	B. Ouslander	0
No. 24				Budrovitch 2	B. Outlander	Budrovitch 2	3200
Fiorella 2	Fiorella 2	Fiorella 2	0	Budrovitch 2	Gerhart	Gerhart	0
Fiorella 2	Fiorella 2	Lasersohn	0	Budrovitch 2	Gerhart	Budrovitch 2	3200
Fiorella 2	Fiorella 2	Stolte	200	Budrovitch 2	Budrovitch 1	Budrovitch 2	3200
Fiorella 2	Fiorella 2	219	0	Budrovitch 2	Budrovitch 3	Budrovitch 2	3200
Fiorella 2	Fiorella 2	Fiorella 1	0	Budrovitch 2	Unabsorbed (control)	Budrovitch 2	3200
Fiorella 2	Lasersohn	Lasersohn	0	No. 27			
Fiorella 2	Lasersohn	Fiorella 2	1600	Budrovitch 3	Budrovitch 1	Budrovitch 3	3200
Fiorella 2	Lasersohn	Fiorella 1	1600	Budrovitch 3	Budrovitch 2	Budrovitch 3	3200
Fiorella 2	Stolte	Stolte	0	Budrovitch 3	Unabsorbed (control)	Budrovitch 3	3200

tests were made. Finally all strains were tested for agglutination with normal rabbit serum. No agglutination occurred in dilutions 1:25 or above.

Small and Dixon¹⁶ studied 10 strains of the influenza bacillus by agglutination and absorption tests. They concluded that this organism can be divided into groups by these methods and that they encountered 4 groups.

Park, Wollstein, and Fleming and Clemenger¹⁷ have been unable to divide the influenza bacillus into groups by agglutination. Valentine

¹⁶ Jour. Infect. Dis., 1920, 26, p. 230.

¹⁷ Lancet, 1919, 2, p. 869.

and Cooper,¹⁸ after employing agglutination and absorption tests, believe that they are dealing with a heterogenous group or organisms with subgroups, and that identical strains are encountered.

CONCLUSIONS

The influenza bacillus represents a heterogenous group of organisms as shown by agglutination and absorption tests. Identical strains occur.

No differentiation can be made by these methods between the organisms isolated from normal healthy throats within two months preceding the epidemic and those isolated from the throats of influenza patients.

A person may carry in the throat three different strains of this organism at the same time.

The morphology alone is unreliable as a means of dividing this group of organisms into subgroups.

¹⁸ Jour. of Immunol., 1919, 4, p. 359.